ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové
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Title of Doctoral Thesis **Analysis of membrane proteins of pathogenic bacterium Francisella tularensis**

Bacterium Francisella tularensis is highly infectious pathogen causing disease tularaemia. Due to the lack of standardization and little protection against highly virulent strains, the only vaccine developed against this pathogen is not allowed for clinical usage. Conserved hypothetical lipoprotein homological to thiol/disulfide oxidoreductase (DsbA) was recently described as essential virulence factor of Francisella tularensis. The dsbA gene deletion led to attenuation of the strain and development of immunoprotection. The DsbA protein sequence revealed the presence of carboxy-terminal DsbA_Com1-like domain harbouring the catalytic active site C-X-X-C and cis-proline and domain amino-terminal to FKBP type peptidyl-prolyl isomerase. This work was focused on functional a substrate characterization of DsbA protein. The functional analysis of this protein showed both the importance of the active site, cis-proline and the FKBP_N domain for the thiol/disulphide oxidoreductase activity. Further, this work also revealed the in vitro chaperone activity of DsbA protein fully dependent on DsbA-Com1-like domain. The possibility of dimerization function of FKBP_N domain was refused and the monomeric state of DsbA protein in bacterial cell was confirmed.
Using the SILAC metabolic labelling the substrate analyses of potential DsbA partners showed 63 proteins with significantly altered abundance in the dsbA deletion strain compared with the wild type. One can assume that some of these proteins are important for F. tularensis virulence.